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Cytokines and nitric oxide synthase inhibitor as mediators of adrenergic refractoriness in cardiac myocytes

Carmine V. Oddis [a,b], Mitchell S. Finkel [c,d,*]

[a] *Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*
[b] *Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*
[c] *Department of Medicine, West Virginia University School of Medicine, Morgantown, WV, USA*
[d] *Department of Pharmacology, West Virginia University School of Medicine, Morgantown, WV, USA*

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Abstract

We have previously proposed that pro-inflammatory cytokines and nitric oxide (NO) contributed to reversible myocardial depression in patients with sepsis and congestive heart failure. Sepsis and heart failure are also associated with refractoriness to β-adrenoceptor agonists. Therefore, the chronotropic effects of cytokines and the NO synthase inhibitor, N^G-methyl-L-arginine (NMA), on β-adrenoceptor stimulation of neonatal cardiac myocytes were studied. Tumor necrosis factor α, interleukin-1β and interleukin-6 but not interleukin-4 or interleukin-5 significantly enhanced spontaneous beating rates compared to untreated myocytes in serum-free media for 48 h ($P < 0.01$; $n = 12$ for each). NMA also significantly enhanced spontaneous beating rates ($P < 0.01$; $n = 12$ for each). Only interleukin-1β treatment resulted in significant nitrite production, immunohistochemical staining for inducible nitric oxide synthase and detection of inducible NO synthase messenger RNA by reverse transcriptase-polymerase chain reaction (RT-PCR). However, tumor necrosis factor α, interleukin-1β, interleukin-6, and NMA each completely blocked the positive chronotropic effects of the β-adrenoceptor agonist, isoproterenol ($P < 0.01$; $n = 12$ for each). These findings are most consistent with an inducible NO synthase-independent effect of cytokines and NMA on the chronotropic responses of neonatal cardiac myocytes to β-adrenoceptor stimulation. This effect of cytokines and NMA on adrenergic signaling may involve a myocardial constitutive NO synthase or an NO-independent mechanism.

Keywords: Inflammation; Heart; Sepsis; Heart failure

1. Introduction

Nitric oxide (NO) is formed from the amino acid L-arginine by a distinct family of NO synthases (Ignarro, 1994; Moncada and Higgs, 1993; Nathan and Xie, 1994). Constitutive NO synthases have been decribed in neural tissue and endothelium (Nathan and Xie, 1994). Pro-inflammatory cytokines induce a third isoform of this enzyme in macrophages, hepatocytes and smooth muscle cells (Geller et al., 1993; Koide et al., 1993; Nussler et al., 1993; Xie et al., 1992). Cardiac myocytes have also been shown to express inducible NO synthase in response to cytokine stimulation (Balligand et al., 1994; Oddis et al., 1995; Oddis et al., 1994; Roberts et al., 1992).

We have previously proposed that pro-inflammatory cytokines and NO contributed to reversible myocardial depression in patients with sepsis and congestive heart failure (Barry, 1994; Nathan and Xie, 1994; Finkel et al., 1993; Oddis et al., 1995). Cytokine-stimulated NO production has been reported to depress cardiac myocyte contractility through activation of soluble guanylate cyclase (Brady et al., 1993; Mery et al., 1993; Shah et al., 1994; Wahler and Dollinger, 1995). The NO-cGMP pathway has been shown to depress sarcolemmal L-type Ca^{2+} channel activity and to decrease the sensitivity of contractile proteins to Ca^{2+} in cardiac myocytes (Mery et al., 1993; Shah et al., 1994; Wahler and Dollinger, 1995).

Reversible myocardial depression observed in sepsis and congestive heart failure is also associated with refractoriness to β-adrenoceptor stimulation (Bristow et al., 1985; Feldman and Bristow, 1990; Hare et al., 1995; Neuman et al., 1988). The underlying mechanism responsible for this adrenergic hyporesponsiveness is unknown. NO has been

* Corresponding author. Department of Medicine, Section of Cardiology, Robert C. Byrd Health Sciences Center, P.O. Box 9156, Morgantown, WV 26506-9156, USA. Tel.: (1-304) 293-4097; fax: (1-304) 293-7828.

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implicated as a modulator of adrenergic signaling in cardiac myocytes (Balligand et al., 1993; Rozanski and Witt, 1994). The present studies were undertaken to determine if cytokines blunt chronotropic effects of adrenergic stimulation by inducing the expression of inducible NO synthase by cardiac myocytes. We report for the first time that pro-inflammatory cytokines and the NO synthase inhibitor, N^G-methyl-L-arginine (NMA), completely blocked the positive chronotropic effect of the β-adrenoceptor agonist, isoproterenol, on neonatal cardiac myocytes in culture.

2. Materials and methods

2.1. Materials

All reagents were purchased from Sigma (St. Louis, MO, USA) unless otherwise indicated.

2.2. Cardiac myocyte studies

Myocytes were prepared from the ventricles of 1–2 day old rat pups as previously described (Oddis et al., 1994). Rats were decapitated immediately following CO_2 anesthesia in accordance with the guidelines of the University of Pittsburgh Animal Care and Use Committee. Briefly, the ventricles of 30–50 hearts were minced in Ca^{2+} and Mg^{2+} free Hank's Balanced Salt Solution (HBSS; Gibco BRL, Grand Island, NY, USA) and digested for 15 min periods in 10 ml of a solution containing 0.1% trypsin (Gibco BRL), 15 units/ml collagenase and 0.1 mg/ml deoxyribonuclease (Worthington Biochemical, Freehold, NJ, USA) in HBSS. Digestion was stopped by adding 10 ml of Dulbecco's Modified Eagles Medium (DMEM/F12; Gibco BRL) containing 5% calf serum. Cycles were repeated until all tissue was digested. The cell suspension was differentially plated to remove fibroblasts, endothelium and other contaminating cell types, followed by further purification of myocytes with a Percoll (Sigma) gradient system in HBSS (Vincent and Nadeau, 1984). The purified myocytes were cultured in DMEM/F12 supplemented with 5% calf serum, penicillin (50 units/ml) and streptomycin (50 μg/ml). Cells were seeded at a density of 1.25×10^5 cells/cm^2 on 48 well plates (Flacon Plastics, Cockeysville, MD, USA; Costar, Cambridge, MA, USA) which were previously coated with a 0.05% collagen solution (Calbiochem, La Jolla, CA, USA) and allowed to dry overnight. This preparation resulted in cradiac myocyte cultures with purities of $\geq 95\%$. Culture medium was changed to fresh serum-free DMEM/F12 containing insulin, transferrin, selenium, linoleic acid and bovine serum albumin (Sigma) 48 h after plating. Myocytes formed confluent monolayers of spontaneously beating cells 24 h later. These cells were washed and fresh serum-free DMEM/F12 was added. Cytokines (Genzyme, Boston, MA, USA) alone or in combination with NMA were added at this time.

Beating rates were determined by counting the time required for 30 beats of the myocytes using an enclosed, heated stage maintained at 37°C. Beating rates remained stable for at least 1 h. Determinations were made after equilibrating for at least 15 min. Data represent the means ± S.E.M. of 12 different determinations derived from 4 wells each from 3 separate myocytes preparations of 30–50 hearts/preparation.

2.3. Assay for nitrite production

Nitrite assays on neonatal rat cardiac myocyte cell culture supernatants were performed as described previously (Oddis et al., 1994). Briefly, the stable end-product of NO synthesis, nitrite, was used as a measure of NO production. Cell culture supernatants were mixed with an equal volume of Greiss reagent for 1 h (1 part 0.1% napthylenediamine dihydrochloride and 1 part 1% sulfanilamide in 5% phosphoric acid; Sigma). The absorbance at 550 nm was measured with a microplate reader (Dynatech Instruments, Torrance, CA, USA). The nitrite concentration was determined by using a sodium nitrite standard curve ranging from 10^{-6}–10^{-4} M. The absorbances from experimental supernatants were compared to known values from the standard curve to determine the nitrite concentrations.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was performed as previously described with modifications (Oddis et al., 1995; Kramnik et al., 1993). Total cellular RNA was isolated by acid guanidinium isothiocyanate-phenol-chloroform extraction (Chomzynski and Sacchi, 1987) RNA was quantified spectrophotometrically at 260 nm. First-strand complementary DNA (cDNA) was generated by adding 1 mM dNTP's, 1 U/μl Rnase inhibitor, 2.5 U/μl reverse transcriptase, 2.5 μM Oligo $d(T)_{16}$ (Perkin Elmer, Foster City, CA, USA) to 5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3 in a final volume of 20 μl. Reverse transcription was carried out at 37°C for 1h. Followed by heat-inactivation of the enzyme at 99°C for 5 min. Polymerase chain reaction (PCR) was then carried out by adding 1 μl of first-strand cDNA to 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM: sense (5′-CCCTTCCGAAGTTTCTGGCAGCAGC-3′), and antisense (5′-GGCTGTCAGAGCCTCGTGGCTTTGG-3′) inducible NO synthase primers (Clontech Laboratories, Palo Alto, CA, USA), and 2 units of Taq polymerase (Perkin Elmer) in a final volume of 50 μl. These samples were overlayed with 75 μl of mineral oil and subjected to 35 cycles of 94°C for 45 s, 65°C for 45 s, and 72°C for 2 min in a DNA thermal cycler (Perkin Elmer). First-strand cDNA was also subjected to PCR using 0.4 μM: β-actin specific sense (5′-TTGTAACCAACTGGGACGATATGG-3′) and antisense

(5′-GATCTTGATCTTCATGGTGCTAGG-3′) primers (Clontech) in the same reaction conditions that were used for inducible NO synthase cDNA detection. These samples were subjected to 35 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 2 min. PCR products were run on 1.8% ethidium bromide agarose gels for 75 min and visualized by exposure to ultraviolet light. Single PCR amplified products of the expected size were obtained for inducible NO synthase (497 bp) and β-actin (764bp). ϕ-X174 RFDNA/*Hae*III fragments (DNA ladder) were used as DNA size markers (Gibco).

2.5. Immunohistochemical staining of iNOS protein

Cardiac myocytes were cultured on Lab-Tek slides (Fisher, Pittsburgh, PA, USA) as described previously (Oddis et al., 1995). Cells were fixed with 2.5% paraformaldehyde for 15 min at room temperature and then treated with triton X-100 (0.2% in phosphate buffered saline) for 10 min. Cells were incubated with normal goat serum for 1 h. After two additional rinses with phosphate buffered saline containing 0.5% bovine serum albumin (Sigma), cells were then incubated with a mouse monoclonal antibody (Transduction Laboratories, Lexington, KY, USA) specific for inducible NO synthase for 2 h. The antibody was generated against a 21 kDa protein fragment corresponding to amino acids 961–1144 of mouse macrophage inducible NO synthase. The antibody was diluted 1:250 prior to use. Cells were then incubated with rhodamine red labeled goat anti-mouse IgG (Sigma) at a dilution of 1:3000 for 1 h. Cells were washed two additional times in phosphate-buffered saline and coverslips were placed on the slides and observed with a Nilkon FXA fluorescent microscope (Nikon).

2.6. Statistical methods

Data represent the means ± S.E.M. of 12 different determinations derived from 4 wells each from 3 separate myocyte preparations of 30–50 hearts/preparation. Analysis of variance (ANOVA) and the Student-Newman-Keuls test were used for multi-group comparisons. Values of $P < 0.05$ were considered statistically significant.

3. Results

The spontaneous beating rates of neonatal cardiac myocytes decreased following exposure to serum-free media for 48 h (153 ± 4 vs. 57 ± 6 beats/min, respectively; $P < 0.01$; $n = 12$). The addition of pro-inflammatory cytokines, tumor necrosis factor α, interleukin-1β or interleukin-6 significantly enhanced spontaneous beating rates compared to vehicle at 48 h (119 ± 19, 102 ± 13, 125 ± 16 vs. 57 ± 6 beats/min, respectively; $P < 0.01$; $n = 12$ for each). Neither interleukin-4 nor interleukin-5 had any effect on spontaneous beating rates compared to vehicle at 48 hs (48 ± 5, 45 ± 10 vs. 57 ± 6 beats/min, respectively; P = ns; $n = 12$ for each).

Only interleukin-1β treatment resulted in an increase in

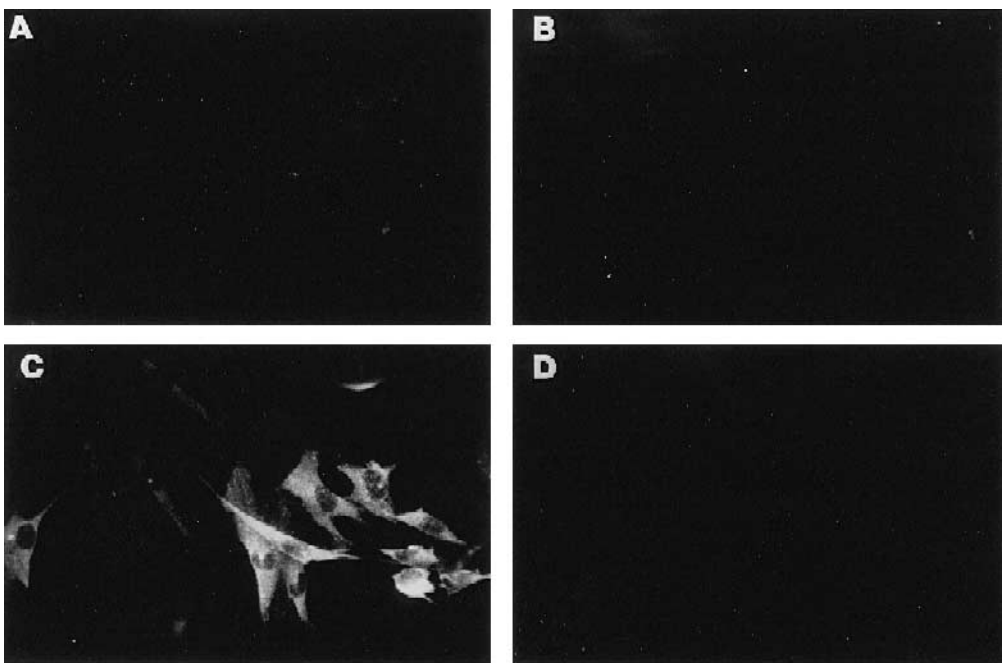

Fig. 1. Representative photomicrographs of cultured neonatal rat cardiac myocytes immunohistochemically stained with a monoclonal antibody against inducible NO synthase (see Section 2). Cells were treated with vehicle (×400) (A), 1000 U/ml tumor necrosis factor α (TNF-α) (×400) (B), 500 U/ml interleukin-1 β (IL-1) (×400) (C), or 1000 U/ml interleukin-6 (IL-6) (×400) (D). Each of the above experiments were repeated three times using three different myocyte cultures with the same results.

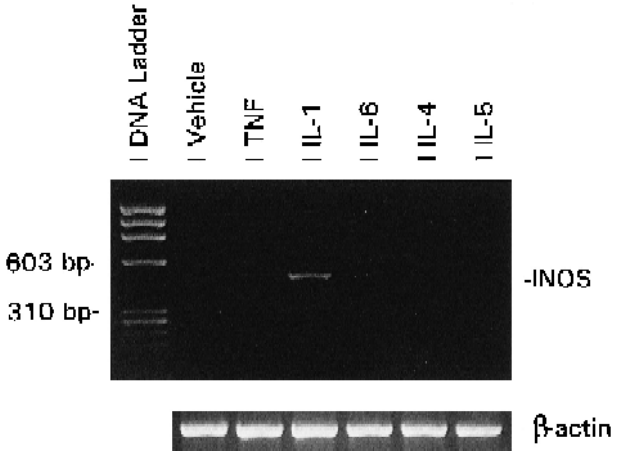

Fig. 2. RT-PCR analyses of the effects of vehicle, tumor necrosis factor α (TNF-α), interleukin-1β (IL-1), interleukin-6 (IL-6), interleukin-4 (IL-4) or interleukin-5 (IL-5) on inducible NO synthase and β-actin messenger RNA abundance in cardiac myocytes. Representative photographs of RT-PCR products from cells treated with vehicle, 1000 U/ml TNF, 500 U/ml IL-1, 1000 U/ml IL-6, 1000 U/ml IL-4 or 100 U/ml IL-5 for 24 h. Each of the above experiments were repeated three times using three different myocyte cultures with the same results.

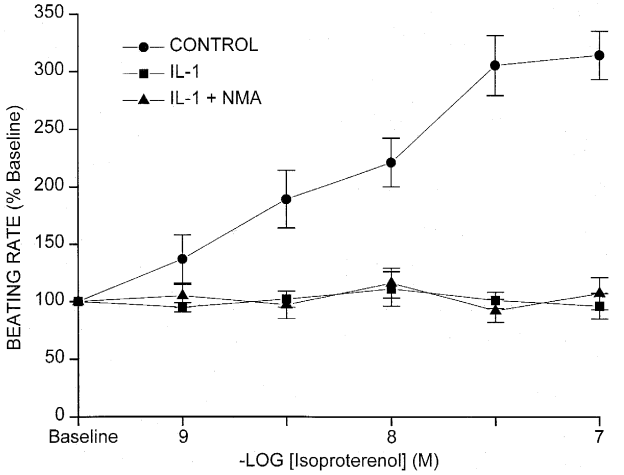

Fig. 4. Graph depicting the chronotropic effects of increasing concentrations of isoproterenol on cells exposed to control conditions, interleukin-1β (IL-1), or IL-1 + NMA for 48 h. Baseline spontaneous beating rates (beats/min) for cells exposed to control conditions (•), 500 U/ml IL-1 (■) or 500 U/ml IL-1 + 10^{-5} M NMA (▲) were 57 ± 6, 102 ± 13 and 125 ± 13, respectively. Values represent the means ± S.E.M. of 4 replicate wells from 3 separate cultures of 30–50 hearts/culture ($n = 12$).

supernatant nitrite compared to vehicle alone (4.2 ± 0.7 vs. 0.2 ± 0.1 nmol/1.25 × 10^5cells per 48 h, respectively; $P < 0.01$; $n = 12$). Tumor necrosis factor α, interleukin-6, interleukin-4 and interleukin-5 had no effect on nitrite production (0.4 ± 0.2, 0.4 ± 0.2, 0.8 ± 0.2, 0.8 ± 0.2 nmol/1.25 × 10^5cells per 48 h, respectively; $n = 12$). Immunohistochemical staining for inducible NO synthase protein revealed that only interleukin-1β-treated myocytes stained positively for inducible NO synthase protein (Fig. 1). RT-PCR revealed the presence of inducible NO synthase messenger RNA in interleukin-1β-treated cardiac myocytes but not in myocytes treated with vehicle, tumor necrosis factor α, interleukin-6, interleukin-4 or interleukin-5 (Fig. 2). RT-PCR revealed no significant changes in β-actin messenger RNA abundance in cardiac myocytes treated under the various experimental conditions (Fig. 2).

Isoproterenol increased the beating rates of neonatal cardiac myocytes in a concentration-dependent manner

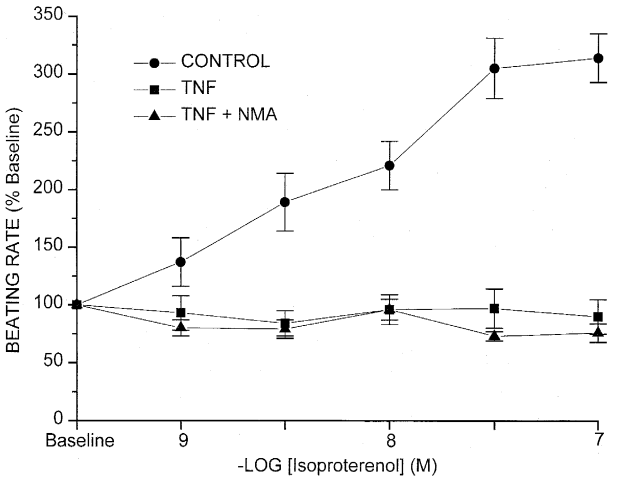

Fig. 3. Graph depicting the chronotropic effects of increasing concentrations of isoproterenol on cells exposed to control conditions, tumor necrosis factor α (TNF) or TNF + NMA for 48 h. Baseline spontaneous beating rates (beats/min) for cells exposed to control conditions (•), 1000 U/ml TNF (■) or 1000 U/ml TNF + 10^{-5} M NMA (▲) were 57 ± 6, 119 ± 19 and 126 ± 11, respectively. Values represent the means ± S.E.M. of 4 replicate wells from 3 separate cultures of 30–50 hearts/culture ($n = 12$).

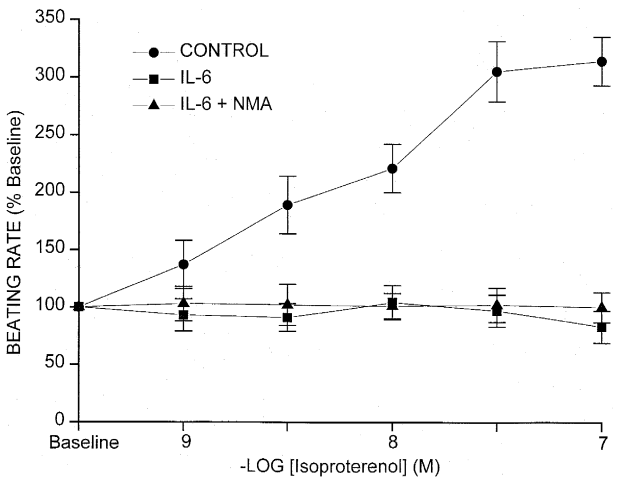

Fig. 5. Graph depicting the chronotropic effects of increasing concentrations of isoproterenol on cells exposed to control conditions, interleukin-6 (IL-6) or IL-6 + NMA for 48 h. Baseline spontaneous beating rates (beats/min) for cells exposed to control conditions (•), 1000 U/ml IL-6 (■) or 1000 U/ml IL-6 + 10^{-5} M NMA (▲) were 57 ± 6, 125 ± 16 and 120 ± 16, respectively. Values represent the means ± S.E.M. of 4 replicate wells from 3 separate sultures of 30–50 hearts/culture ($n = 12$).

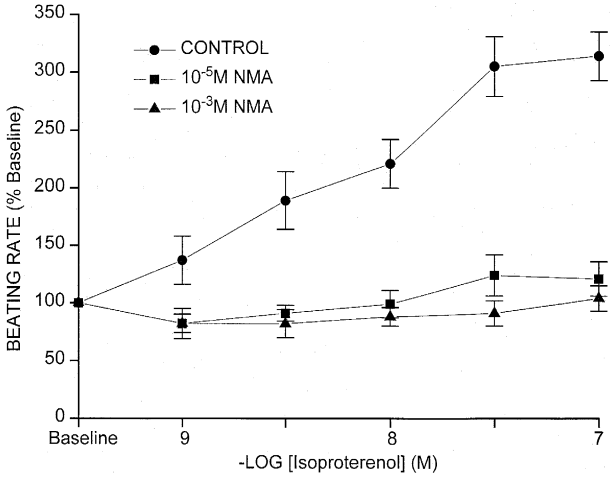

Fig. 6. Graph depicting the chronotropic effects of increasing concentrations of isoproterenol on cells exposed to control conditions, 10^{-5} M NMA or 10^{-3} M NMA for 48 h. Baseline spontaneous beating rates (beats/min) for cells exposed to control conditions (•), 10^{-5} M NMA (■) or 10^{-3} M NMA (▲) were 57 ± 6, 101 ± 14 and 114 ± 15, respectively. Values represent the means ± S.E.M. of 4 replicate wells from 3 separate cultures of 30–50 hearts/culture ($n = 12$).

($E_{max} = 314 \pm 21\%$ at 10^{-7} M; $n = 12$) (Figs. 3, 4 Figs. 56). Pre-treatment of myocytes with tumor necrosis factor α for 48 h completely blocked the positive chronotropic effect of isoproterenol ($P < 0.01$; $n = 12$) (Fig. 3). NMA had no effect on tumor necrosis factor α inhibition of the chronotropic effects of isoproterenol ($P =$ ns; $n = 12$) (Fig. 3). Pre-treatment of myocytes with interleukin-1β for 48 h also blocked the positive chronotropic effect of isoproterenol ($P < 0.01$; $n = 12$) (Fig. 4). NMA had no effect on interleukin-1β inhibition of the isoproterenol effect ($P =$ ns; $n = 12$) (Fig. 4). Pre-treatment with interleukin-6 for 48 h also blocked the positive chronotropic effect of isoproterenol ($P < 0.01$; $n = 12$) (Fig. 5). NMA had no effect on interleukin-6 inhibition of the isoproterenol effect ($P =$ ns; $n = 12$) (Fig. 5). Pre-treatment with 10^{-5} M NMA or 10^{-3} M NMA for 48 h also completely blocked the positive chronotropic effect of increasing concentrations of isoproterenol ($P < 0.01$; $n = 12$ for each) (Fig. 6). When the spontaneous beating rates for cardiac myocytes treated with the maximal isoproterenol concentration (10^{-7} M) were compared for all conditions, only control conditions without NMA were significantly increased over the other conditions except for interleukin-1β + NMA ($P < 0.05$; $n = 12$) (Fig. 7). Interleukin-1β + NMA was significantly different than interleukin-1β without NMA and tumor necrosis factor α + NMA ($P < 0.05$; $n = 12$) (Fig. 7).

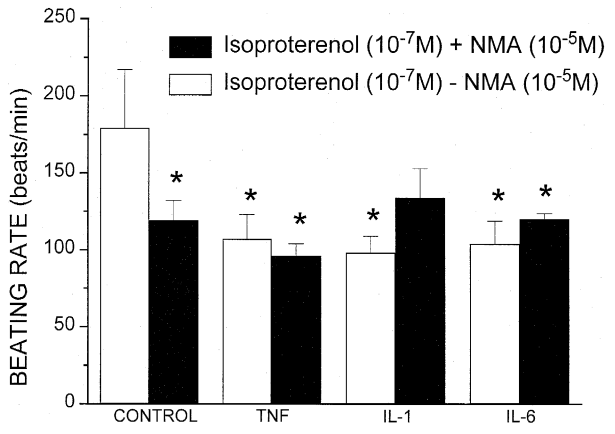

Fig. 7. Graph depicting the chronotropic effects of 10^{-7} M isoproterenol on cells exposed to control conditions ± NMA, tumor necrosis factor α (TNF-α) ± NMA, interleukin-1β (IL-1) ± NMA or interleukin-6 (IL-6) ± NMA (119 ± 13, 179 ± 35, 96 ± 8, 107 ± 16, 134 ± 19, 98 ± 11, 120 ± 4 and 104 ± 15 beats/min, respectively). * $P < 0.05$ vs. Control without NMA. Values represent the means ± S.E.M. of 4 replicate wells from 3 separate cultures of 30–50 hearts/culture ($n = 12$).

4. Discussion

We and others have provided evidence that pro-inflammatory cytokines and NO reversibly depressed myocardial contractility (Brady et al., 1993; DeBelder et al., 1993; Finkel et al., 1992, 1993; Mery et al., 1993; Wahler and Dollinger, 1995). We have previously reported that 10^{-5} M NMA blocked the negative inotropic effect of pro-inflammatory cytokines in isolated hamster papillary muscle preparations (Finkel et al., 1992). We further demonstrated that 10^{-5} M NMA altered the force-frequency relationship in isolated hamster papillary muscles (Finkel et al., 1995). These studies using micromolar concentrations of NMA in isolated hamster papillary muscle preparations provided indirect, presumptive evidence for the existence of a functional constitutive NO synthase in the myocardium. Other investigators have provided further support for the existence of a constitutive myocardial NO synthase (DeBelder et al., 1993; Moncada and Higgs, 1993). Thus, the acute inotropic effects of pro-inflammatory cytokines, NMA, NO and myocardial constitutive NO synthase have been implicated in the same signal transduction pathways in the heart.

The results of our present study in neonatal cardiac myocytes contrasts sharply with our previous results using the papillary muscle preparation. The acute (within minutes) inotropic effects of cytokines were blocked by NMA in the papillary muscle preparation. The longer term (48 h) chronotropic effects of cytokines were shared by NMA in the cardiac myocyte culture system. The spontaneous beating rates of neonatal cardiac myocytes decreased over 48 hours in serum free media ($P < 0.01$; $n = 12$). This decrease in the beating rate was blocked by the addition of NMA, as well as tumor necrosis factor α, interleukin-1β or interleukin-6 ($P < 0.01$; $n = 12$ for each). The specificity of this effect was supported by the failure of interleukin-4 or interleukin-5 to similarly maintain spontaneous beating rates ($P < 0.01$; $n = 12$ for each).

β-Adrenoceptor stimulation with isoproterenol resulted

in a concentration-dependent increase in beating rates (Figs. 3–6). Pre-treatment of cardiac myocytes for 48 h with pro-inflammatory cytokines or NMA completely blocked the positive chronotropic effect of β-adrenoceptor stimulation with isoproterenol (Figs. 3–6). The combination of cytokines and NMA also completely blocked the positive chronotropic effect of isoproterenol (Figs. 3–6).

The refractoriness to adrenergic stimulation caused by pro-inflammatory cytokines and NMA is clearly not related to induction of inducible NO synthase. Only interleukin-1β treatment resulted in increased nitrite levels, inducible NO synthase messenger RNA and immunohistochemical staining for inducible NO synthase protein (Figs. 1 and 2) ($P < 0.01$; $n = 12$). The lower level of detectibility of the Greiss reagent is approximately 1 μM (Green et al., 1982). This precludes drawing definitive conclusions regarding the effects of these agents on submicromolar concentrations of NO that could result from stimulation or inhibition of myocardial constitutive NO synthase. These studies also cannot exclude the possibility of intracellular translocation of NO into subcellular compartments by cytokines and NMA. The direct measurement of NO with a porphyrinic microsensor will be required to definitively determine the role of myocardial constitutive NO synthase in these chronotropic effects of cytokines and NMA (Balligand et al., 1994).

The addition of 10^{-7} M isoproterenol significantly enhanced cardiac myocyte spontaneous beating rates in control conditions over each of the other conditions except for interleukin-1β + NMA (Fig. 7). These results suggest that cytokines and NMA have not saturated the isoproterenol signal transduction pathway. Therefore, isoproterenol could further increase beating rates over those achieved with cytokine and NMA treatment. The mechanisms responsible for catecholamine-induced β-adrenoceptor desensitization have been extensively studied (Benovic et al., 1988; Ungerer et al., 1994; Shih and Malbou, 1994). Tumor necrosis factor α has been shown to cause an increase in $G_{i\alpha}$ and G_{β} proteins and adenylyl cyclase responsiveness in neonatal rat cardiac myocytes (Reithman et al., 1991). Several different regulatory components of the adrenergic signaling pathway have been identified as potential sites for desensitization (Benovic et al., 1988; Ungerer et al., 1994; Shih and Malbou, 1994). Adrenergic stimulation with catecholamines has also been shown to result in blunting non-adrenergic responses (heterologous desensitization) (Benovic et al., 1988). Cytokines and NMA illustrate the invrese of this property with catecholamines, i.e., adrenergic desensitization following non-adrenergic stimulation. Tumor necrosis factor α has been shown to suppress the positive inotropic and chronotropic effects of β-adrenoceptor agonists as well as the positive inotropic and arrhythmogenic effects of β-adrenoceptor agonists (Oddis et al., 1994; Reithman and Werdan, 1994). This may imply that the main effect of tumor necrosis factor α, and possibly interleukin-1β and interleukin-6, is on a final common pathway of second messengers rather than on the β-adrenoceptor axis or α-adrenoceptor pathway (Yokoyama et al., 1994).

The mechanism(s) responsible for the adrenergic refractoriness resulting from exposure to cytokines and NMA must be related to the signal transduction pathways involved in adrenergic modulation of neonatal myocyte beating rates. This signal transduction pathway clearly involves binding of β-adrenoceptor agonists to the β-receptor, activation of a stimulatory G-protein (G_s), conversion of ATP to cAMP through adenylate cyclase and phosphorylation of sarcolemmal L-type Ca^{2+} channels (Katz, 1992). We have previously reported that NMA had no effect on dihydropyridine binding to sarcolemmal L-type Ca^{2+} channels or ryanodine binding to sarcoplasmic reticulum Ca^{2+} release channels (Finkel et al., 1995). This makes the direct binding to these sites very unlikely candidates for a mechanism of action of cytokines and NMA on neonatal myocyte beating rates. Indirect effects on these sites are entirely reasonable, however. Alternative possibilities include down regulation of β-adrenoceptors, inhibition of G_s, inhibition of adenylate cyclase, increased cGMP, enhancement of G_i, enhancement of phosphodiesterase activity and non-specific membrane stabilizing effects. Considerably more work will be needed to identify and fully characterize the molecular mechanisms by which cytokines and NMA modulate adrenergic responsiveness in neonatal cardiac myocytes.

We report for the first time that both cytokines and NMA blocked adrenergic responses in neonatal cardiac myocytes. Refractoriness to β-adrenoceptor stimulation is characteristic of clinical sepsis and congestive heart failure (Bristow et al., 1985; Feldman and Bristow, 1990; Hare et al., 1995; Neuman et al., 1988). Evidence is rapidly accumulating supporting a role for pro-inflammatory cytokines and NO in the reversible myocardial depression observed in these conditions (Barry, 1994; Brady et al., 1992; Finkel et al., 1992; Oddis et al., 1995). Dobutamine significantly increased heart rate, cardiac index and left ventricular stroke work index in septic patients compared with septic shock patients (Silverman et al., 1993). Langendorff perfused hearts from septic rats had significantly increased basal beating rates and were more responsive to lower concentrations of isoproterenol (Smith et al., 1986). These investigators concluded that β-adrenergic chronotropic sensitivity is increased in sepsis. These results are in contrast with the results of this study using neonatal rat cardiac myocytes in culture. The disparities could be due to the fact that the studies by Silverman et al. (1993) and Smith et al. (1986) were conducted in patients and whole organ, respectively, versus cardiac myocytes in culture. The cytokine concentration could also account for the differences in results. Local cytokine concentrations could be much lower (or higher) in the septic hearts also accounting for the disparity in results. The results of the present study support a role for pro-inflammatory cy-

tokines in β-adrenoceptor hyporesponsiveness, as well. The considerable clinical and fundamental implications of this interaction between the neuro-humoral and immune modulation of cardiac function justifies further investigation.

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